No. 200800079

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Agriculture & Agri-Food Canada

Whereas, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE PURPOSES, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT. IN THE UNITED STATES SEED OF THIS VARIETY (1) MAY BE SOLD BY VARIETY NAME ONLY AS A CLASS OF CERTIFIED SEED AND (2) SHALL CONFORM TO THE NUMBER OF GENERATIONS SPECIFIED BY THE OWNER OF THE RIGHTS. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

WHEAT, DURUM

'Strongfield'

In Testimony Whereof, *I have hereunto set my hand and caused the seal of the* Plant Variety Protection Office *to be affixed at the City of Washington, D.C. this seventh day of April, in the year two thousand and eight.*

Attest:

Commissioner
Plant Variety Protection Office
Agricultural Marketing Service

Secretary of Agriculture

BUREAU OF ENGRAVING AND PRINTING

REPRODUCE LOCALLY. *Include form number and date on all reproductions*

Form Approved - OMB No. 0581-0055

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE AND TECHNOLOGY - PLANT VARIETY PROTECTION OFFICE

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE
(Instructions and information collection burden statement on reverse)

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

1. NAME OF OWNER
Agriculture & Agri-Food Canada

2. TEMPORARY DESIGNATION OR EXPERIMENTAL NAME
DT712

3. VARIETY NAME
Strongfield

4. ADDRESS *(Street and No., or R.F.D. No., City, State, and ZIP Code, and Country)*
Ann de St. Remy, PhD
Lacombe Research Centre
6000 C & E Trail; Lacombe, AB; T4L 1W1

5. TELEPHONE *(include area code)*
(403) 782-8126

6. FAX *(include area code)*
(403) 782-6120

FOR OFFICIAL USE ONLY

PVPO NUMBER
200800079

FILING DATE
Jan. 25, 2008

7. IF THE OWNER NAMED IS NOT A "PERSON", GIVE FORM OF ORGANIZATION *(corporation, partnership, association, etc.)*
Government of Canada

8. IF INCORPORATED, GIVE STATE OF INCORPORATION

9. DATE OF INCORPORATION

FEES RECEIVED

FILING AND EXAMINATION FEES:
$ 4382.00
DATE 1-25-2008

CERTIFICATION FEE:
$ 768.00
DATE 3/18/08

10. NAME AND ADDRESS OF OWNER REPRESENTATIVE(S) TO SERVE IN THIS APPLICATION. *(First person listed will receive all papers)*
Dale Clark, Director of Research
Westbred LLC
81 Timberline Drive
Bozeman, Montana 59718-6994

11. TELEPHONE (Include area code)
406-587-1218

12. FAX (Include area code)
406-586-8247

13. E-MAIL
dclark@westbred.com

14. CROP KIND *(Common Name)*
Durum Wheat

16. FAMILY NAME *(Botanical)*
Poaceae

15. GENUS AND SPECIES NAME OF CROP
Triticum turgidum

17. IS THE VARIETY A FIRST GENERATION HYBRID?
☐ YES ☒ NO

18. DOES THE VARIETY CONTAIN ANY TRANSGENES? *(OPTIONAL)*
☐ YES ☒ NO
IF SO, PLEASE GIVE THE ASSIGNED USDA-APHIS REFERENCE NUMBER FOR THE APPROVED PETITION TO DEREGULATE THE GENETICALLY MODIFIED PLANT FOR COMMERCIALIZATION. _____

19. CHECK APPROPRIATE BOX FOR EACH ATTACHMENT SUBMITTED
(Follow instructions on reverse)

a. ☒ Exhibit A. Origin and Breeding History of the Variety
b. ☒ Exhibit B. Statement of Distinctness
c. ☒ Exhibit C. Objective Description of Variety
d. ☐ Exhibit D. Additional Description of the Variety *(Optional)*
e. ☒ Exhibit E. Statement of the Basis of the Owner's Ownership
f. ☒ Exhibit F. Declaration Regarding Deposit
g. ☒ Voucher Sample *(3,000 viable untreated seeds or, for tuber propagated varieties, verification that tissue culture will be deposited and maintained in an approved public repository)*
h. ☒ Filing and Examination Fee ($4,382), made payable to "Treasurer of the United States" *(Mail to the Plant Variety Protection Office)*

20. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE SOLD ONLY AS A CLASS OF CERTIFIED SEED? *(See Section 83(a) of the Plant Variety Protection Act)*
☒ YES *(If "yes", answer items 21 and 22 below)*
☐ NO *(If "no", go to item 23)*
☐ UNDECIDED

21. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF CLASSES?
☐ YES ☒ NO
IF YES, WHICH CLASSES? ☐ FOUNDATION ☐ REGISTERED ☐ CERTIFIED

22. DOES THE OWNER SPECIFY THAT SEED OF THIS VARIETY BE LIMITED AS TO NUMBER OF GENERATIONS?
☐ YES ☒ NO
IF YES, SPECIFY THE NUMBER 1,2,3, etc. FOR EACH CLASS.
☐ FOUNDATION ☐ REGISTERED ☐ CERTIFIED
(If additional explanation is necessary, please use the space indicated on the reverse.)

23. HAS THE VARIETY (INCLUDING ANY HARVESTED MATERIAL) OR A HYBRID PRODUCED FROM THIS VARIETY BEEN SOLD, DISPOSED OF, TRANSFERRED, OR USED IN THE U. S. OR OTHER COUNTRIES?
☒ YES ☐ NO Canada 2005: US March 2007
IF YES, YOU MUST PROVIDE THE DATE OF FIRST SALE, DISPOSITION, TRANSFER, OR USE FOR EACH COUNTRY AND THE CIRCUMSTANCES. *(Please use space indicated on reverse.)*

24. IS THE VARIETY OR ANY COMPONENT OF THE VARIETY PROTECTED BY INTELLECTUAL PROPERTY RIGHT *(PLANT BREEDER'S RIGHT OR PATENT)*?
☑ YES ☐ NO
IF YES, PLEASE GIVE COUNTRY, DATE OF FILING OR ISSUANCE AND ASSIGNED REFERENCE NUMBER. *(Please use space indicated on reverse.)*

25. The owners declare that a viable sample of basic seed of the variety has been furnished with application and will be replenished upon request in accordance with such regulations as may be applicable, or for a tuber propagated variety a tissue culture will be deposited in a public repository and maintained for the duration of the certificate.

The undersigned owner(s) is(are) the owner of this sexually reproduced or tuber propagated plant variety, and believe(s) that the variety is new, distinct, uniform, and stable as required in Section 42, and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act.

Owner(s) is (are) informed that false representation herein can jeopardize protection and result in penalties.

SIGNATURE OF OWNER		SIGNATURE OF OWNER	
NAME *(Please print or type)*		NAME *(Please print or type)*	
John Culley, PhD, Director			
CAPACITY OR TITLE	DATE	CAPACITY OR TITLE	DATE
Office of Intellectual Property & Commercialization	February 14, 2008		

200800079

(See reverse for instructions and information collection burden statement)

GENERAL INSTRUCTIONS: To be effectively filed with the Plant Variety Protection Office (PVPO), **ALL** of the following items must be **received** in the PVPO: (1) Completed application form signed by the owner; (2) completed exhibits A, B, C, E, F; (3) for a tuber reproduced variety, verification that a viable *(in the sense that it will reproduce an entire plant)* tissue culture will be deposited and maintained in an approved public repository; and (4) payment by credit card or check drawn on a U.S. bank for $4,382 ($518 filing fee and $3,864 examination fee), payable to "Treasurer of the United States" *(See Section 97.6 of the Regulations and Rules of Practice)*. **NEW:** With the application for a seed reproduced variety **or by direct deposit soon after filing**, the applicant must provide at least 3,000 viable untreated seeds of the variety *per se*, and for a hybrid variety at least 3,000 untreated seeds of each line necessary to **reproduce** the variety. Partial applications will be held in the PVPO for not more than 90 days; then returned to the applicant as un-filed. Mail application and other requirements to Plant Variety Protection Office, AMS, USDA, Room 401, NAL Building, 10301 Baltimore Avenue, Beltsville, MD 20705-2351. Retain one copy for your files. All items on the face of the application are self explanatory unless noted below. Corrections on the application form and exhibits must be initialed and dated. **DO NOT** use masking materials to make corrections. If a certificate is allowed, you will be requested to send a payment by credit card or check payable to "Treasurer of the United States" in the amount of $768 for issuance of the certificate. Certificates will be issued to owner, not licensee or agent.

NOTES: It is the responsibility of the applicant/owner to keep the PVPO informed of any changes of address or change of ownership or assignment or owner's representative during the life of the application/certificate. The fees for filing a change of address; owner's representative; ownership or assignment; or any modification of owner's name is specified in Section 97.175 of the regulations. *(See Section 101 of the Act, and Sections 97.130, 97.131, 97.175(h) of the Regulations and Rules of Practice.)*

Plant Variety Protection Office
Telephone: (301) 504-5518 **FAX:** (301) 504-5291
General E-mail: PVPOmail@usda.gov
Homepage: http://www.ams.usda.gov/science/pvpo/PVPindex.htm

SPECIFIC INSTRUCTIONS:
To avoid conflict with other variety names in use, the applicant must check the appropriate recognized authority and **provide evidence** that the permanent name of the application variety (even if it is a parental, inbred line) has been cleared by the appropriate recognized authority before the Certificate of Protection is issued. For example, for agricultural and vegetable crops, contact: U.S. Department of Agriculture, Agricultural Marketing Service, Livestock and Seed Programs, **Seed Regulatory and Testing Branch**, 801 Summit Crossing Place, Suite C, Gastonia, North Carolina 28054-2193 Telephone: (704) 810-8870. http://www.ams.usda.gov/lsg/seed.htm.

ITEM

19a. Give:
(1) the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method;
(2) the details of subsequent stages of selection and multiplication;
(3) evidence of uniformity and stability; and
(4) the type and frequency of variants during reproduction and multiplication and state how these variants may be identified

19b. Give a summary of the variety's distinctness. Clearly state how this application variety may be distinguished from all other varieties in the same crop. If the new variety is most similar to one variety or a group of related varieties:

(1) identify these varieties and state all differences objectively;
(2) attach replicated statistical data for characters expressed numerically and demonstrate that these are clear differences; and
(3) submit, if helpful, seed and plant specimens or photographs (prints) of seed and plant comparisons which clearly indicate distinctness.

19c. Exhibit C forms are available from the PVPO Office for most crops; specify crop kind. Fill in Exhibit C (Objective Description of Variety) form as completely as possible to describe your variety.

19d. Optional additional characteristics and/or photographs. Describe any additional characteristics that cannot be accurately conveyed in Exhibit C. Use comparative varieties as is necessary to reveal more accurately the characteristics that are difficult to describe, such as plant habit, plant color, disease resistance, *etc.*

19e. Section 52(5) of the Act requires applicants to furnish a statement of the basis of the applicant's ownership. An Exhibit E form is available from the PVPO.

20. If "Yes" is specified *(seed of this variety be sold by variety name only, as a class of certified seed)*, the applicant **MAY NOT** reverse this affirmative decision after the variety has been sold and so labeled, the decision published, or the certificate issued. However, if "No" has been specified, the applicant may change the choice. *(See Regulations and Rules of Practice, Section 97.103).*

23. *See Sections 41, 42, and 43 of the Act and Section 97.5 of the regulations for eligibility requirements.*

24. *See Section 55 of the Act for instructions on claiming the benefit of an earlier filing date.*

22. CONTINUED FROM FRONT *(Please provide a statement as to the limitation and sequence of generations that may be certified.)*

23. CONTINUED FROM FRONT *(Please provide the date of first sale, disposition, transfer, or use for each country and the circumstances, if the variety (including any harvested material) or a hybrid produced from this variety has been sold, disposed of, transferred, or used in the U.S. or other countries.)*

Canada 2005 ; USA March 2007

24. CONTINUED FROM FRONT *(Please give the country, date of filing or issuance, and assigned reference number, if the variety or any component of the variety is protected by intellectual property right (Plant Breeder's Right or Patent).)*

Country: Canada; Grant of Rights Date: 2005-05-13; Certificate Number:2105

Strongfield
Durum spring wheat

#200800079

19.a. Exhibit A Origin and Breeding History

'Strongfield' spring durum wheat (*Triticum turgidum* L. *var. durum*) (Reg. no. CV-1000, PI 641223) was developed at the Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, Swift Current, SK, and received registration No.5819 from the Canadian Food Inspection Agency on 18 May 2004. It was released because of its superior agronomic performance, end-use quality attributes, and reduced grain cadmium concentration. Strongfield was granted Plant Breeder's Rights by the Canadian Food Inspection Agency, certificate no. 2105, on 13 May 2005.

Strongfield was selected from the cross 'AC Avonlea'/DT665 made in 1994 and was developed using a modified pedigree breeding method. DT665 derives from the cross 'Kyle'/'Nile'; Nile was obtained from the International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria. The F_2 generation was grown in 1995 as individual plants in a nursery inoculated with leaf (caused by *Puccinia triticina* Eriks.) and stem rust (caused by *Puccinia graminis* Pers.:Pers. f.sp. *tritici* Eriks. & E. Henn.). Individual spikes from selected plants were grown in $F_{2:3}$ single 3 m rows near Swift Current in 1996. The $F_{3:4}$ and $F_{5:6}$ generations were grown as head rows in a winter nursery near Christchurch, New Zealand to produce seed for yield tests. Unreplicated $F_{3:5}$ and $F_{5:7}$ yield trials were grown near Swift Current and Regina, Saskatchewan and Lethbridge, Alberta in 1997 and 1998 and selected for agronomic performance, disease resistance, and quality (protein, pigment, and gluten strength). An $F_{5:8}$ line designated 9468-CL5 was evaluated in pre-registration trials in 1999 (five locations), and under the designation DT712 in the Durum Cooperative Test in 2000 to 2002 (10 to 12 locations per year).

Each year stem and leaf rust were evaluated in inoculated field trials near Winnipeg, Manitoba using mixtures of prevalent races. The stem rust races used were: QFC (C75), QTH (C25), TPM (C53), TMR (C10), TMR (C95), RTH (C57), RRQ (C63), and RKQ (C63). The races of leaf rust used were MCDS, MBDS, MBR, MBRJ, MGB, TJB, TJBJ, TGBJ, and 128–1 (74–2). Races L1, L16, T1, T6, T13, and T19 of common bunt [caused by *Tilletia laevis* Kuhn in Rabenh., and *T. tritici* (Bjerk.) G. Wint. in Rabenh.] were used for screening of the Durum Cooperative Test entries in inoculated field trials near Lethbridge, Alberta. Strongfield is resistant to prevalent leaf rust, stem rust, and common bunt races. It is susceptible to loose smut [caused by *Ustilago tritici* (Pers.) Rostr.] races T32 and T33, and resistant to race T26, the races prevalent in western Canada.

Strongfield was tested in the Montana State University statewide yield trials in 2006 and 2007 (Tables 1. and 2.) Grain from these trials was used to measure end use quality traits of Strongfield grown in Montana (Table 3), and the quality was found to be similar to the check varieties.

Strongfield has the low cadmium allele described by Clark et. al (1997), see attached, also see Table 4, which reduces grain cadmium (heavy metal) concentration to about half that of the checks.

200800079

Breeder seed, originating from 133 $F_{5:10}$ Breeder lines, will be maintained by the Seed Increase Unit of Agriculture and Agri-Food Canada, Indian Head, SK. S0G 2K0. Distribution and multiplication of Select, Foundation, Registered, and Certified seed stocks will be handled by SeCan, 201–52 Antares Drive, Ottawa, Ontario, K2E 7Z1.

Foundation seed of Strongfield was produced in Canada in 2004. Registered seed was produced in Canada in 2005. The first unencumbered sale of Strongfield in Canada was in March 2006. Certified seed was first produced in the US in 2006 and the first unencumbered sale of Strongfield in the US occurred in March 2007.

A variant that is similar to Strongfield, but is 4 to 8 inches taller, occurs at a frequency of up to .06% (6 per 10,000 plants). Also, a non-glaucous plant variant may occur at a frequency of up to 0.03% (3 per 10,000 plants). Otherwise, Strongfield is a stable and uniform variety in appearance and performance across several generations (F4 to F11) and growing conditions.

19.b. Exhibit B Statement of Distinctness

Strongfield is most like the variety AC Avonlea, however Strongfield carries the low cadmium allele described by Clarke et. al (1997), which reduces grain cadmium concentration to about half that of the checks, and AC Avonlea does not contain this gene, see Table 4.

Clarke, J.M., D. Leisle, and G.L. Kopytko. 1997. Inheritance of cadmium concentrations in five durum wheat crosses. Crop Sci. 37:1722-1726. (see attached)

The above comparison, along with the complete Objective Description (Exhibit C), shows Strongfield to be a distinct variety of durum spring wheat.

Strongfield
Durum spring wheat

#200800079

Table 1. 2006 Montana State University statewide durum trial summary (10 locations).

entry	Heading	Height, cm	Test wt, lb/bu	Grain protein,	hvac	color	1000-seed wt, gm	Disease rating, %*	sawfly damage, %*	Yield, bu/ac
Strongfield	62.9	81.5	57.9	16.08	87.3	23.6	32.9	0.0	0.3	43.7
AC Avonlea	60.6	83.7	58.6	14.75	91.1	23.3	37.0	1.5	0.7	46.7
Alzada	58.6	71.7	58.2	13.93	82.2	23.9	40.0	2.2	0.7	43.1
Alkabo	61.4	82.0	58.9	14.86	84.8	23.1	35.4	1.0	1.6	45.6
Dilse	62.5	82.7	59.1	14.81	85.3	23.3	34.8	0.3	1.8	43.9
Divide	62.9	85.0	58.5	14.94	81.8	23.4	35.2	0.0	0.3	46.1
Grenora	61.3	78.6	58.2	14.65	81.8	24.7	35.1	0.0	1.3	46.6
Kyle	63.8	93.9	58.3	14.82	85.0	22.8	33.7	0.0	3.0	43.0
Maier	61.2	81.8	58.6	15.12	83.7	23.2	34.4	0.3	1.3	44.2
Mountrail	62.0	78.9	57.8	15.12	80.0	25.0	32.4	0.7	1.7	46.1
Average	61.1	81.5	58.2	14.80	82.4	23.5	34.2	0.9	1.0	44.6

*two sites only

Table 2. 2007 Montana State University statewide durum trial summary (9 locations).

entry	Heading*	Height, cm	Test wt, lb/bu	Grain protein, %	hvac	1000-seed wt, gm	Yield, bu/ac, all sites	Yield, bu/ac, dryland sites	Yield, bu/ac, irrigated sites
Strongfield	65.0	82.6	59.2	14.67	80.1	32.6	47.5	42.6	64.4
Alzada	62.4	70.7	58.9	13.66	77.9	36.6	47.2	45.0	54.5
Alkabo	64.1	81.5	60.5	14.08	76.9	34.8	49.5	44.5	66.8
Mountrail	64.9	82.1	59.1	14.42	73.8	32.0	50.1	44.1	71.2
Divide	65.2	84.9	59.6	13.85	75.1	33.9	46.7	42.0	63.1
Pierce	64.9	85.5	60.2	14.36	78	32.0	45.7	39.9	65.7
Grenora	64.1	79	59.5	14.15	78.9	34.1	50.4	44.5	71.4
Levante	63.3	62.6	59	13.63	69.1	33.0	46.5	43.3	57.6
Average	64.3	72.4	59.3	14.09	76.1	33.0	46.3	42.2	60.7

* days from planting

#200800079

Table 3. Quality analysis of Strongfield compared to check varieties in 2006 Montana State University

VARIETY	TW lb/bu	1000 kwt (g)	Grain Protein 12mb%	Semolina Extraction %	Semolina Color L	Semolina Color b	Semolina Protein 12mb%	Semolina Ash 14mb%	Mixogram Pattern
Strongfield	58.0	30.8	16.1	54.5	79.2	24.2	15.9	0.838	5.2
AC Avonlea	59.0	34.4	15.5	55.7	79.8	24.5	14.7	0.819	2.9
Kyle	58.6	30.7	15.4	54.8	79.7	23.1	14.6	0.815	2.7
Alzada	58.8	36.6	14.8	56.9	79.4	25.9	13.8	0.825	6.6

Table 4. Cadmium levels of Strongfield compared to check varieties in Montana State University trials at Conrad, Montana in 2006.

SetDurumICP-03-06.xls ug/g

Site	Cultivar	Cd2265	rank
CONRAD DRY	AC AVONLEA	0.333	1
CONRAD DRY	LEBSOCK	0.313	2
CONRAD DRY	ALZADA	0.306	3
CONRAD DRY	MONROE	0.287	4
CONRAD DRY	KYLE	0.271	5
CONRAD DRY	MOUNTRAIL	0.250	6
CONRAD DRY	**STRONGFIELD**	**0.160**	**7**

Jung, G.A., J.A. Shaffer, and W.L. Stout. 1988. Switchgrass and big bluestem responses to amendments on strongly acid soil. Agron. J. 80:669–676.

Murray, G.M., B.J. Scot, Z. Hochman, and B.J. Butler. 1987. Failure of liming to increase grain yield of wheat and triticale in acid soils may be due to the associated increase in incidence of take-all (*Gaeumannomyces graminis* var. *tritici*). Aust. J. Exp. Agric. 27:411–417.

Troeh, F.R., and L.M. Thompson. 1993. Phosphorus. p. 215–234. *In* Soils and soil fertility. 5th ed. Oxford University Press, Inc., New York.

Unruh, L., and D. Whitney. 1986. Soil acidity and aluminum toxicity: An important factor in winter wheat yields. Better Crops Plant Food 70(Summer):20–22.

Westerman, R.L. 1987. Soil reaction-acidity, alkalinity, and salinity. p. 340–344. *In* E.G. Heyne (ed.) Wheat and wheat improvement. 2nd ed. Agron. Monogr. 13. ASA, CSSA, and SSSA, Madison, WI.

Inheritance of Cadmium Concentration in Five Durum Wheat Crosses

J. M. Clarke,* D. Leisle, and G. L. Kopytko

ABSTRACT

High cadmium concentration in cereal grains has been cited as a human health concern. Several reports showed that grain cadmium concentration was higher in durum (*Triticum turgidum* L. var. *durum*) than in common wheat (*T. aestivum* L.). The objective of this research was to determine the inheritance of observed differences in grain cadmium concentration of durum wheat. This information could be used to facilitate breeding of cultivars with low grain cadmium concentration. Grain cadmium concentration was determined in the F_2 and in $F_{2:3}$ families of one cross and in $F_{2:3}$ and $F_{3:4}$ families of two crosses. Grain and leaf cadmium concentration was measured in random $F_{8:9}$ and $F_{8:10}$ families of three crosses. All trials were conducted in the field on Aridic Haploborall, Vertic Cryoborall, or Gleyed Black soils. Grain cadmium concentration was largely controlled by a single gene, with low cadmium dominant. Leaf cadmium concentration was highly correlated with grain cadmium concentration (r = 0.87–0.89, $P <$ 0.01). Therefore, leaf cadmium concentration can predict the plant phenotype, which would be useful in backcrossing the low cadmium trait into high cadmium cultivars. Heritability in standard units, estimated by $F_{2:3}$ progeny regression on F_2 parent or $F_{2:4}$ progeny regression on $F_{2:3}$ parent, ranged from 0.84 ± 0.06 to 0.88 ± 0.08, indicating that breeding of low grain cadmium cultivars is feasible. Heritability estimated from variance components (years, locations, replications, and genotypes) was 0.78, with 90% confidence limits of 0.88 and 0.67. The simple inheritance and high heritability of grain cadmium concentration will facilitate the breeding of low cadmium concentration durum cultivars.

HIGH LEVELS of cadmium in food crops are a concern in human diets because of possible negative effects on health (Wagner, 1993). Cereal grains represent a large portion of our diet and are thus a major contributor to cadmium intake (Wagner, 1993). This has prompted governments to legislate maximum cadmium concentrations permissible in grain and grain products. For example, the limit for cereal grain products excluding bran is 50 ng g^{-1} in Australia (Oliver et al., 1994), and 60 ng g^{-1} in non-oilseed sunflower (*Helianthus annuus* L.) in Germany (Li et al., 1995b).

J.M. Clarke, Semiarid Prairie Agric. Res. Centre, Agriculture and Agri-Food Canada, Swift Current, SK. S9H 3X2; D. Leisle (retired), Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Rd., Winnipeg, MB. R3T 2M9; and G.L. Kopytko, Dept. Of Biology, Univ. of Massachusetts, 100 Morrissey Blvd., Boston, MA 02125. Received 8 Nov. 1996. *Corresponding author (clarkej@em.agr.ca).

Published in Crop Sci. 37:1722–1726 (1997).

While genetic manipulation of cadmium levels has been limited, there are reports of genetic variation in several economic crops. For example, Hinesly et al. (1978) found variation in leaf and grain cadmium concentration of corn (*Zea mays* L.) inbreds, and Crews and Davies (1985) reported genetic variation for cadmium concentration of lettuce (*Lactuca sativa* L.). Recently, Li et al. (1995b) reported a large range in kernel cadmium concentration of sunflower, and this variation is being exploited to develop low cadmium hybrids (Li et al., 1995a). Genotypic variation in grain cadmium concentration has been reported in both common (Oliver et al., 1995) and durum wheat (Penner et al., 1995).

Research in Australia showed that cadmium level in common wheat varied with site (Oliver et al., 1995) and preceding crop (Oliver et al., 1993). Grain cadmium concentration of wheat was higher following lupin (*Lupinus* spp.) than following cereals (Oliver et al., 1993). Levels of cadmium were higher in durum than in common wheat (Meyer et al., 1982). Zook et al. (1970) reported an average cadmium concentration of 130 ng g^{-1} in durum wheat, compared with 70 to 100 ng g^{-1} in common wheat.

Large genotypic differences in grain cadmium concentration of durum wheat (Penner et al., 1995) suggest the possibility of breeding cultivars with low grain cadmium concentration. Detailed knowledge of the inheritance of the trait would facilitate such a breeding program. The objective of this study was to provide this information by determining the inheritance of cadmium concentration in durum crosses derived from diverse low grain cadmium concentration parents.

MATERIALS AND METHODS

The inheritance of cadmium concentration was investigated in five durum crosses under field conditions near Swift Current, Saskatchewan, on a Aridic Haploborall (sandy loam) (Ayres et al., 1985), near Indian Head, Saskatchewan, on a Vertic Cryoborall (fine, mixed), or near Glenlea, Manitoba, on a Gleyed Black Soil.

Crosses and Experiments

'Fanfarron'/DT 369. Fanfarron (PI221411) is of Yugoslavian origin, and DT 369 (PI546362; McLeod et al., 1991b) is a semidwarf line from the Swift Current breeding program. Approximately 150 random F_2 seeds and 40 seeds of each of

the parents were space-planted in single 3-m rows in 1992. Rows were separated by rows of spring-planted winter wheat; row spacing was 0.23m and seeding rate was approximately 30 seeds per m^2. In 1993, 50 random $F_{2:3}$ selections and the parents were grown in single rows in a randomized complete block design with two replications. Row configuration was the same as 1992, and seeding rate was approximately 70 seeds per m^2. Grain cadmium concentration was determined on 77 F_2 plants in 1992, and on all plots in 1993.

Random $F_{8:9}$ families of this cross were developed from material grown as natural selection bulks from F_2 through F_7. The F_2 consisted of 1600 plants, and each generation thereafter was propagated from 1600 seeds drawn randomly from bulk-harvested seed from the previous generation. Single F_8 seeds were planted in a winter nursery near Brawley, CA, for multiplication. In 1993, 31 $F_{8:9}$ families were grown in single rows arranged in a randomized complete block design with two replications. Row configuration was as described above, and seeding rate was 300 seeds per m^2. Bulk harvested seed ($F_{8:10}$) from the 1993 test was used to plant a randomized complete block design with two replications at Swift Current in 1994. The plots consisted of four rows 3 m long, with a row spacing of 0.23 m and a seeding rate of 300 seeds per m^2. Plots were separated by a row of spring-planted winter wheat. Grain cadmium concentration was determined on a composite sample of each line in 1993 (data not reported), and on all plots in 1994. Cadmium concentration of above-ground plant material sampled at the four- to five-leaf stage was determined in 1994.

'Tschernovska'/DT 369. Tschernovska (PI278444) originated in the former USSR. Thirty-nine random $F_{8:9}$ families, developed in the same manner as the Fanfarron/DT 369 families, were grown at Swift Current in 1993 and 1994 as described above. Grain cadmium concentration was determined in 1993 (data not reported), and cadmium concentration of grain and vegetative material were determined in 1994.

STD40/DT 369. The line STD40 was obtained from ICARDA, Aleppo, Syria. Thirty-three random $F_{8:9}$ families, developed in the same manner as the Fanfarron/DT 369 families, were grown at Swift Current in 1993 and 1994 as described above. Grain cadmium concentration was determined in 1993 (data not reported), and cadmium concentration of grain and vegetative material was determined in 1994.

'Kyle'/'Nile'. Kyle (PI537310; McLeod et al., 1991a) is from the Swift Current breeding program, and Nile was obtained from ICARDA. Forty-two random $F_{2:3}$ families were grown in a randomized complete block design with two replications at Swift Current and Indian Head in 1993. Bulk-harvested seed ($F_{2:4}$) from the Swift Current test was used to repeat the trial at both locations in 1994. Plots consisted of single 3-m rows separated by rows of spring-planted winter wheat. Row spacing was 0.23 m, and seeding rate was 300 seeds per m^2. Grain cadmium concentration was determined on all plots in both years.

'Sceptre'/'Biodur'. Sceptre is a Canadian cultivar (Knott, 1986), and Biodur is a French cultivar. One hundred forty-eight random $F_{2:3}$ families and the parents were grown in 1992 in an unreplicated trial at Glenlea, Manitoba. Plots consisted of single rows 2.1 m long, with a spacing of 0.3 m; the seeding rate was approximately 60 seeds per m^2. Grain cadmium was determined on all lines. In 1993, 105 unreplicated $F_{2:4}$ families were sown at Glenlea from bulk-harvested seed of the $F_{2:3}$ families from the 1992 trial. Grain cadmium concentration data were collected from 87 lines.

Cadmium Analysis

Oven-dry grain or plant samples (5 g) were ground with a laboratory mill with stainless steel blades, and 0.5 g was digested in 6 mL trace-metal grade $HNO_3/HClO_4$ (2:1 v/v) for 16 h at room temperature. The samples were then digested in a block digester for 60 min at 100°C, 10 min at 120°C, and 45 min at 220°C. A reagent blank and certified reference sample (Nat. Inst. of Standards and Technology, Gaithersburg, MD) was processed with each set of 40 samples. Cadmium was determined on a 20-μL aliquot with a Hitachi Z8200 (Hitachi Scientific Instruments, Nissel Sagyo Canada Inc., Rexdale, ON) flame-furnace atomic absorption spectrophotometer with polarized Zeeman background correction in graphite tubes.

Data Analysis

Narrow-sense heritability for the Fanfarron/DT 369 cross was calculated by regressing $F_{2:3}$ family means on F_2 plant values. In the Sceptre/Biodur cross, $F_{2:4}$ family grain cadmium was regressed on $F_{2:3}$ family values. Standardized data were used in both cases (Frey and Horner, 1957). For testing of genetic ratios, F_2 plants with cadmium concentration greater than one standard error of the mean below the high parent (DT 369) were considered to be high cadmium, and all others were assumed to be low cadmium. In the random $F_{8:10}$ families, the mid-parent cadmium concentration was used to separate high and low classes. Heritability in the Kyle/Nile cross was calculated from variance components (Comstock and Moll, 1963). The variances were estimated by restricted maximum likelihood (Searle et al., 1992). The calculations were made with SAS PROC MIXED (SAS Institute, 1992) assuming years, locations, replications, and genotypes were random. Exact 90% confidence intervals were calculated after Knapp et al. (1985).

RESULTS AND DISCUSSION

Distributions for the F_2 plants and $F_{2:3}$ families of the Fanfarron/DT 369 cross suggests that grain cadmium concentration is largely controlled by a single gene, with low cadmium dominant (Fig. 1). The F_2 data fit the expected 3 low : 1 high phenotypic ratio for a single gene expressing full dominance (Table 1). Cadmium concentration of the parents and lines was greater in the F_3 than in the F_2, presumably due to different environmental conditions. Factors such as high precipitation (Andersson and Pettersson, 1981), low soil pH (Li et al., 1995), and low availability in the soil of chemically-similar metals such as zinc (Oliver et al., 1994) tend to increase cadmium uptake by plants. Regression of standardized $F_{2:3}$ family means on standardized F_2 plant values for the Fanfarron/DT 369 cross gave a narrow-sense heritability estimate of 0.82 ± 0.08. Selection on a single plant basis will thus be effective in reducing grain cadmium. Narrow-sense heritability for the Sceptre/Biodur cross was 0.84 ± 0.06 (data not shown).

Data from random inbred lines showed a generally bimodal distribution for high and low cadmium (Fig. 2) and fit the expected 1:1 phenotypic ratio (Table 1), supporting the hypothesis of a single gene. The families were tested with a random amplified polymorphic DNA marker linked with the high cadmium allele (Penner et al., 1995) to confirm the scoring of the phenotypic classes. Four of the Fanfarron/DT 369 families with low cadmium phenotype showed presence of the marker, and the marker was absent in one high cadmium family.

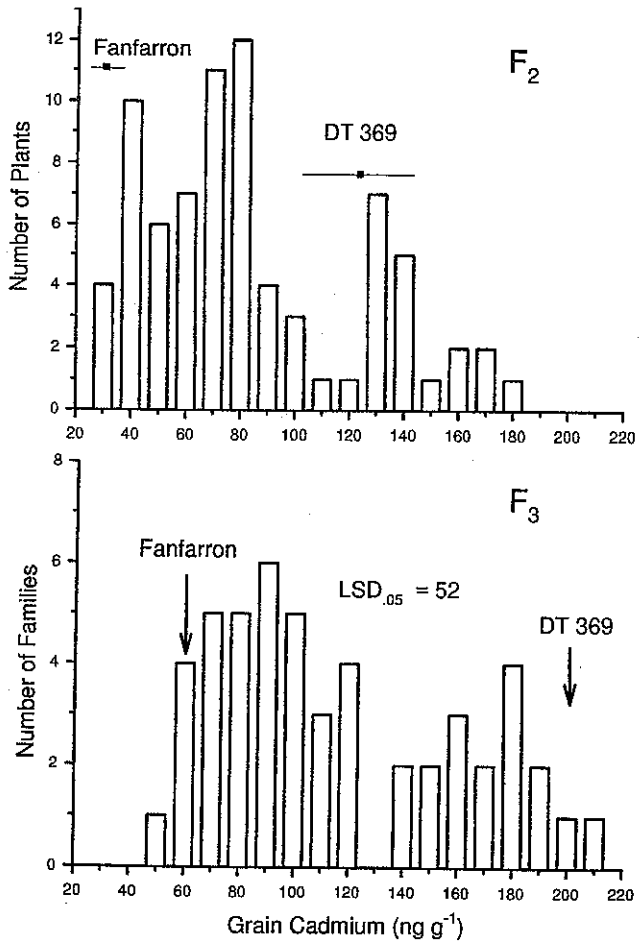

Fig. 1. Grain cadmium concentration of 77 F_2 plants (1992) and 50 $F_{2:3}$ families (1993) from the cross Fanfarron/DT 369 grown at Swift Current. Symbols or arrows show the parental means, and horizontal bars are the standard error of the mean.

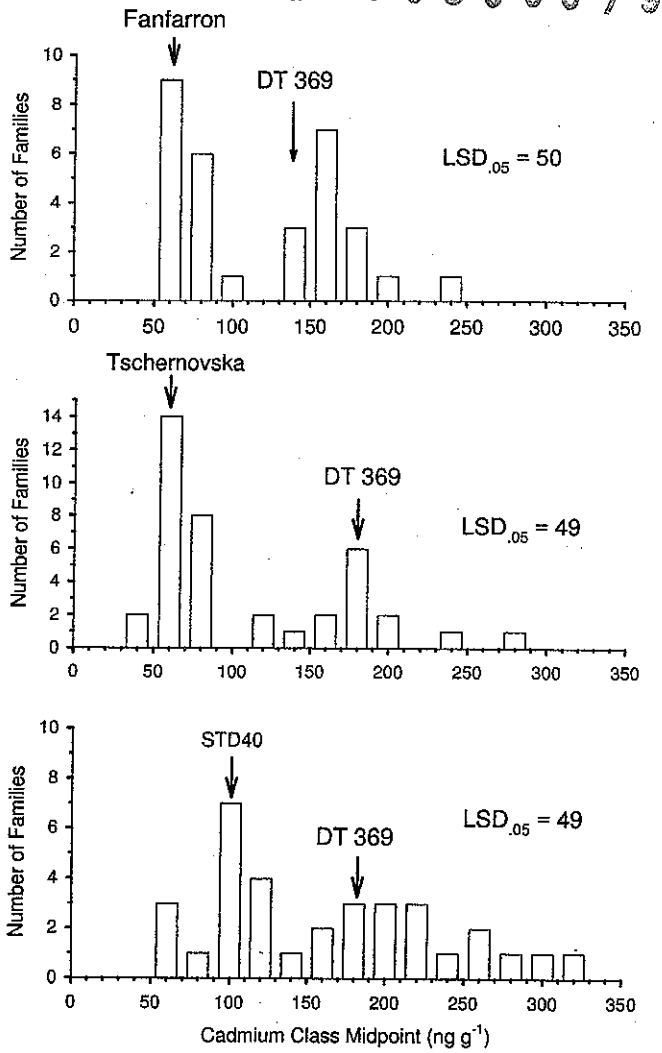

Fig. 2. Grain cadmium concentration of random $F_{8:10}$ lines from the crosses Fanfarron/DT 369 (n = 31), Tschernovska/DT 369 (n = 39), and STD 40/DT 369 (n = 33) grown at Swift Current in 1994.

Similarly, the marker was present in five low cadmium families in the Tschernovska/DT 369 cross. There were no discrepancies for the STD40/ DT 369 cross. Discrepancies between the marker and the phenotype could arise from recombination or misclassification of the phenotype. Apparent transgressive segregation in all three crosses (Fig. 2) indicates the possibility of other minor genes influencing cadmium concentration. Transgressive segregation, misclassification of lines, or small sample size may have contributed to the marginal probability for the 1:1 phenotypic ratio in the Fanfarron/DT 369 cross. Grain cadmium concentration of STD40 was greater ($P < 0.05$) than the other low parents, Fanfarron and Tschernovska. STD40 may have a different gene or genes affecting grain cadmium concentration.

Distribution of leaf cadmium concentration in the inbred lines was also generally bimodal (Fig. 3), but again there was apparent transgressive segregation in all three crosses. The data fit the expected 1:1 pheno-

Table 1. Test of goodness of fit to a single gene dominance model for observed grain and leaf cadmium concentrations of the three durum wheat crosses.

Cross	Generation	Plant part	Expected ratio	Observed number†		χ^2	P
				Low	High		
Fanfarron/DT 369	F_2	grain	3:1	58	19	0.004	0.9–0.95
	$F_{8:10}$	grain	1:1	16	15	0.03	0.7–0.9
		leaf	1:1	16	15	0.03	0.7–0.9
Tschernovska/DT 369	$F_{8:10}$	grain	1:1	24	15	2.08	0.1–0.2
		leaf	1:1	25	14	3.10	0.05–0.1
STD40/DT 369	$F_{8:10}$	grain	1:1	15	18	0.27	0.5–0.7
		leaf	1:1	16	17	0.03	0.7–0.9

† F_2 plants with cadmium concentration greater than one standard error of the mean below the high parent (DT 369) were considered high cadmium; mid-parent cadmium value was used to separate low and high categories for the $F_{8:10}$ families.

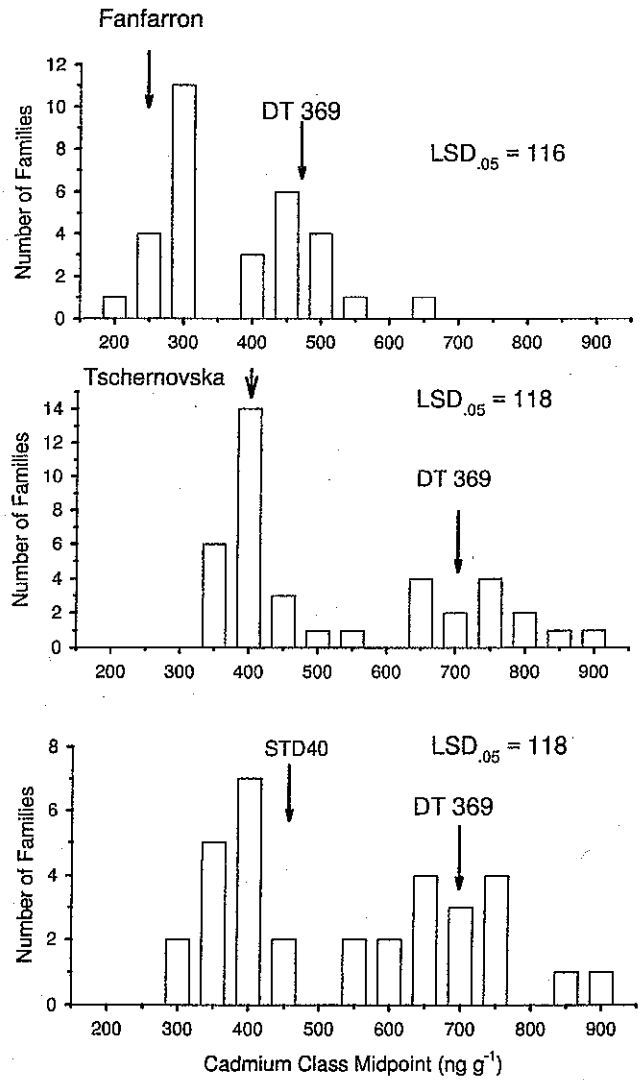

Fig. 3. Leaf cadmium concentration of random $F_{8:10}$ lines from the crosses Fanfarron/DT 369 (n = 31), Tschernovska/DT 369 (n = 39), and STD 40/DT 369 (n = 33) grown at Swift Current in 1994.

typic ratio (Table 1) for a single gene. Leaf and grain cadmium concentration were highly correlated for the three crosses (r = 0.89 for Fanfarron/DT 369 and Tschernovska/DT 369, r = 0.87 for STD40/DT 369; $P <$ 0.01). Phenotypic classification of families for leaf and grain cadmium were the same except for one family in the Fanfarron/DT 369 cross and one in the Tschernovska/DT 369 cross. This indicates that the same or tightly-linked gene(s) control cadmium concentration in vegetative material and grain. It is thus possible to screen plants at the four-leaf stage for grain cadmium phenotype, which would be useful in backcrossing programs. Higher cadmium concentration in leaf tissue than in grain was also observed in corn (Hinesly et al., 1978).

Table 2. Estimates of variance components and heritability (h^2) of grain-cadmium concentration for 42 $F_{2:3}$ and $F_{2:4}$ families of the cross Kyle/Nile grown at Swift Current and Indian Head, Saskatchewan, in 1993 and 1994.

Component†	Variance	SE or CI
σ^2	564	163
$\hat{\sigma}^2_{GY}$	137	60
$\hat{\sigma}^2_{GL}$	49	41
$\hat{\sigma}^2_{GYL}$	0	–
$\hat{\sigma}^2_E$	506	50
h^2	0.78	0.67–0.88‡

† G = genotype, Y = year, L = location, E = error.
‡ 95% confidence interval for h^2.

Table 3. Mean grain cadmium concentration of 42 $F_{2:3}$ (1993) and $F_{2:4}$ (1994) families from the cross Kyle/Nile grown at Swift Current and Indian Head, Saskatchewan.

Location	Entry	Year 1993	Year 1994
		— ng g^{-1} —	
Indian Head	$F_{2:3}$ or $F_{2:4}$ families	109	57
	Kyle	183	64
	Nile	58	44
Swift Current	$F_{2:3}$ or $F_{2:4}$ families	108	65
	Kyle	162	156
	Nile	80	42
SED†	Families-Year	5	
	-Location × Year	7	
	Parents-Loc × Yr × Genotype	23	

† Standard error of a difference.

Heritability of grain cadmium concentration on an entry-mean basis was 0.78 for the Kyle/Nile cross (Table 2). This confirms the single location results for Fanfarron/DT 369 and Sceptre/Biodur showing that grain cadmium concentration is highly heritable. Average cadmium concentration of the families was similar for locations within years, but was higher in 1993 than in 1994 (Table 3). Cadmium concentration of the low parent Nile was much lower than Kyle except at Indian Head in 1994, presumably due to differences in growing conditions.

Further research is required to determine if transgressive segregation for cadmium concentration is caused by other minor genes directly or indirectly affecting cadmium uptake. Indirect effects could result from transpiration rate, which has been shown to influence plant concentration of mineral ions in several species (Masle et al., 1992), or gene(s) affecting uptake of other ions. In oat (*Avena sativa* L.), for example, Mench and Fargues (1994) observed that uptake of cadmium was greater for an iron-efficient cultivar than for an iron-inefficient cultivar, and attributed the difference to exudation of organic acids from the roots of the iron-efficient cultivar. The presence of minor genes affecting cadmium concentration directly or indirectly would not greatly affect breeding strategies for development of low grain cadmium durum cultivars. Of more concern is whether low cadmium genotypes will show reduced uptake of other essential nutrients.

In conclusion, grain cadmium concentration of durum wheat is highly heritable, both on a single plant and family-mean basis, and could be easily manipulated by plant breeding. Cadmium concentration is largely controlled by a single gene, with low cadmium dominant, in the five diverse low cadmium genotypes evaluated in this study.

ACKNOWLEDGMENTS

The assistance of chemists G.E. Winkleman (Semiarid Prairie Agricultural Research Centre) and E. Gawalko (Grain

Research Laboratory, Canadian Grain Commission) with cadmium analyses, and the technical assistance of L. Thiessen, M. Olfert, P. Taylor, and C. Knockert (Semiarid Prairie Agricultural Research Centre) and of A. Romanow (Cereal Research Centre) is gratefully acknowledged.

REFERENCES

Andersson, A., and O. Pettersson. 1981. Cadmium in Swedish winter wheat: Regional differences and their origin. Swedish J. Agric. Res. 11:49–55.

Ayres, K.W., D.F. Acton, and J.G. Ellis. 1985. The soils of the Swift Current Map Area [72] Saskatchewan. Extension Div., Univ. of Saskatchewan, Saskatoon.

Comstock, R.E., and R.H. Moll. 1963. Genotype-environment interactions. p. 164–194. *In* W.D. Hanson and H.F. Robinson (ed.) Statistical genetics and plant breeding. Nat. Acad. of Science - Nat. Res. Council, Washington, DC.

Crews, H.M., and B.E. Davies. 1985. Heavy metal uptake from contaminated soils by six varieties of lettuce (*Lactuca sativa* L.). J. Agric. Sci. (Cambridge) 105:591–595.

Frey, K.J., and T. Horner. 1957. Heritability in standard units. Agron J. 49:59–62.

Hinesly, T.D., D.E. Alexander, E.L. Ziegler, and G.L. Barrett. 1978. Zinc and Cd accumulation by corn inbreds grown on sludge amended soil. Agron. J. 70:425–428.

Knapp, S.J., W.W. Stroup, and W.M. Ross. 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. 25:192–194.

Knott, D.R. 1986. Sceptre durum wheat. Can. J. Plant Sci. 66:407–408.

Li, Y-M., R.L. Chaney, A.A. Schneiter, and J.F. Miller. 1995a. Combining ability and heterosis estimates for kernel cadmium level in sunflower. Crop Sci. 35:1015–1019.

Li, Y-M., R.L. Chaney, A.A. Schneiter, and J.F. Miller. 1995b. Genotypic variation in kernel cadmium concentration in sunflower germplasm under varying soil conditions. Crop Sci. 35:137–141.

Masle, J., G.D. Farquahar, and S.C. Wong. 1992. Transpiration ratio and plant mineral content are related among genotypes of a range of species. Aust. J. Plant Physiol. 19:709–721.

McLeod, J.G., T.F. Townley-Smith, R.M. DePauw, J.M. Clarke, C.W.B. Lendrum, and G.E. McCrystal. 1991a. Registration of 'Kyle' durum wheat Crop Sci. 31:236–237.

McLeod, J.G., T.F. Townley-Smith, R.M. DePauw, J.M. Clarke, C.W.B. Lendrum, and G.E. McCrystal. 1991b. Registration of DT 369 high-yielding, durum wheat germplasm. Crop Sci. 31:1717.

Mench, M.J., and S. Fargues. 1994. Metal uptake by iron-efficient and inefficient oats. Plant Soil 165:227–233.

Meyer, M.W., F.L. Fricke, G.G.S. Holmgren, J. Kubota, and R.L. Chaney. 1982. Cadmium and lead in wheat grain and associated surface soils of major wheat production areas of the United States. p. 34. *In* Agronomy abstracts. ASA, Madison, WI.

Oliver, D.P., J.E. Schultz, K.G. Tiller, and R.H. Merry. 1993. The effect of crop rotations and tillage practices on cadmium concentration in wheat grain. Aust. J. Agric. Res. 44:1221–1234.

Oliver, D.P., R. Hannam, K.G. Tiller, N.S. Wilhelm, R.H. Merry, and G.D. Cozens. 1994. The effects of zinc fertilization on cadmium concentration in wheat grain. J. Environ. Qual. 23:705–711.

Oliver, D.P., J.W. Gartrell, K.G. Tiller, R. Correll, G.D. Cozens, and B.L. Youngberg. 1995. Differential responses of Australian wheat cultivars to cadmium concentration in wheat grain. Aust. J. Agric. Res. 46:873–886.

Penner, G.A., J. Clarke, L.J. Bezte, and D. Leisle. 1995. Identification of RAPD markers linked to a gene governing cadmium uptake in durum wheat. Genome 38:543–547.

Searle, S.R., G. Casella, and C.E. McCullock. 1992. Variance components. John Wiley and Sons, New York.

SAS Institute. 1992. SAS technical report, SAS/STAT software changes and enhancements, Release 6.07. SAS Institute Inc., Cary, NC.

Wagner, G.J. 1993. Cadmium in crops and effects on human health. Adv. Agron. 51:173–212.

Zook, E.G., F.E. Greene, and E.R. Morris. 1970. Nutrient composition of selected wheats and wheat products. VI. Distribution of manganese, copper, nickel, zinc, magnesium, lead, tin, cadmium, chromium, and selenium as determined by atomic absorption spectroscopy and colorimetry. Cereal Chem. 47:720–731.

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE AND TECHNOLOGY
PLANT VARIETY PROTECTION OFFICE
BELTSVILLE, MD 20705

Exhibit C

OBJECTIVE DESCRIPTION OF VARIETY
Wheat (*Triticum* spp.)

NAME OF APPLICANT (S)	TEMPORARY OR EXPERIMENTAL DESIGNATION	VARIETY NAME
SeCan		Strongfield

ADDRESS (Street and No. or RD No., City, State, Zip Code and Country)

561-300 March Road
Kanata, Ontario K2K 2E2
Canada

FOR OFFICIAL USE ONLY

PVPO NUMBER

#200800079

PLEASE READ ALL INSTRUCTIONS CAREFULLY:

Place the appropriate number that describes the varietal character of this variety in the boxes below. Place a zero in the first box (e.g., [0][9][9] or [0][9]) when number is either 99 or less or 9 or less respectively. Data for quantitative plant characters should be based on a minimum of 100 plants. Comparative data should be determined from varieties entered in the same trial. Royal Horticultural Society or any recognized color standard may be used to determine plant colors; designate system used: _____. Please answer all questions for your variety; lack of response may delay progress of your application.

1. KIND:

[2]
1 = Common
2 = Durum
3 = Club
4 = Other (Specify) _____

2. VERNALIZATION:

[1]
1 = Spring
2 = Winter
3 = Other (Specify) _____

3. COLEOPTILE ANTHOCYANIN:

[2] 1 = Absent 2 = Present

4. JUVENILE PLANT GROWTH:

[3] 1 = Prostrate 2 = Semi-Erect 3 = Erect

5. PLANT COLOR: (boot stage)

2 [2]
1 = Yellow-Green
2 = Green
3 = Blue-Green

6. FLAG LEAF: (boot stage)

[1] 1 = Erect 2 = Recurved
[1] 1 = Not Twisted 2 = Twisted
[1] 1 = Wax Absent 2 = Wax Present

7. EAR EMERGENCE:

[0][6][3] Number of Days (Average)
[ ][ ] Number of Days Earlier Than * _____
Same As * _____
[0][4] Number of Days Later Than * Alzada

*Relative to a PVPO-Approved Commercial Variety Grown in the Same Trial

8. ANTHER COLOR:

[1] 1 = Yellow 2 = Purple

Strongfield

#200800079

9. PLANT HEIGHT: (from soil to top of head, excluding awns)

[0][8][2] cm (Average)

[1][0] cm Taller Than Alzada *

Same As _____ *

[ ][ ] cm Shorter Than _____ *

10. STEM:

A. ANTHOCYANIN

[1] 1 = Absent 2 = Present

B. WAXY BLOOM

[2] 1 = Absent 2 = Present

C. HAIRINESS (last internode of rachis)

[2] 1 = Absent 2 = Present

D. INTERNODE

[2] 1 = Hollow 2 = Semi-Solid 3 = Solid

[5] Number of Nodes

E. PEDUNCLE

[1] 1 = Erect 2 = Recurved 3 = Semi-Erect

[3][5] cm Length

F. AURICLE

[1] Anthocyanin: 1 = Absent 2 = Present

[1] Hair: 1 = Absent 2 = Present

11. HEAD: (At Maturity)

A. DENSITY

[3]
1 = Lax
2 = Middense (Laxidense)
3 = Dense

B. SHAPE

[2]
1 = Tapering
2 = Strap
3 = Clavate
4 = Other (Specify) _____

C. CURVATURE

[1]
1 = Erect
2 = Inclined
3 = Recurved

D. AWNEDNESS

[4]
1 = Awnless
2 = Apically Awnletted
3 = Awnletted
4 = Awned

12. GLUMES: (At Maturity)

A. COLOR

[1]
1 = White
2 = Tan
3 = Other (Specify) _____

B. SHOULDER

[2]
1 = Wanting 2 = Oblique
3 = Rounded 4 = Square
5 = Elevated 6 = Apiculate
7 = Other (Specify) _____

C. SHOULDER WIDTH

[1]
1 = Narrow
2 = Medium
3 = Wide

D. BEAK

[3]
1 = Obtuse
2 = Acute
3 = Acuminate

E. BEAK WIDTH

[1]
1 = Narrow
2 = Medium
3 = Wide

F. GLUME LENGTH

[3]
1 = Short (ca. 7 mm)
2 = Medium (ca. 8 mm)
3 = Long (ca. 9 mm)

G. WIDTH

[3]
1 = Narrow (ca. 3 mm)
2 = Medium (ca. 3.5 mm)
3 = Wide (ca. 4 mm)

H. PUBESCENCE

[1]
1 = Not Present
2 = Present

Strongfield

#200800079

13. SEED:

A. SHAPE

[3] 1 = Ovate
2 = Oval
3 = Elliptical

B. CHEEK

[2] 1 = Rounded
2 = Angular

C. BRUSH

[1] 1 = Short
2 = Medium
3 = Long

[1] 1 = Not Collared
2 = Collared

D. CREASE

[2] 1 = Width 60% or less of Kernel
2 = Width 80% or less of Kernel
3 = Width Nearly as Wide as Kernel

[2] 1 = Depth 20% or less of Kernel
2 = Depth 35% or less of Kernel
3 = Depth 50% or less of Kernel

E. COLOR

[2] 1 = White
2 = Amber
3 = Red
4 = Other (Specify) _____

F. TEXTURE

[1] 1 = Hard
2 = Soft
3 = Other (Specify) _____

G. PHENOL REACTION (See Instructions)

[1] 1 = Ivory
2 = Fawn
3 = Light Brown
4 = Dark Brown
5 = Black

H. SEED WEIGHT

[3][3] g/1000 Seed (whole number only)

I. GERM SIZE

[3] 1 = Small
2 = Midsize
3 = Large

14. DISEASE: PLEASE INDICATE THE SPECIFIC RACE OR STRAIN TESTED

(0 = Not Tested 1 = Susceptible 2 = Resistant 3 = Intermediate 4 = Tolerant)

[4] Stem Rust (*Puccinia graminis* f. sp. *tritici*)
[0] Stripe Rust (*Puccinia striiformis*)
[0] Tan Spot (*Pyrenophora tritici-repentis*)
[0] Halo Spot (*Selenophoma donacis*)
[0] *Septoria nodorum* (Glume Blotch)
[0] *Septoria avenae* (Speckled Leaf Disease)
[0] *Septoria tritici* (Speckled Leaf Blotch)
[0] Scab (*Fusarium* spp.)
[0] "Black Point" (Kernel Smudge)
[0] Barley Yellow Dwarf Virus (BYDV)
[0] Soilborne Mosaic Virus (SBMV)
[0] Wheat Yellow (Spindle Streak) Mosaic Virus
[0] Wheat Streak Mosaic Virus (WSMV)
[0] Other (Specify) _____
[0] Other (Specify) _____
[0] Other (Specify) _____

[4] Leaf Rust (*Puccinia recondita* f. sp. *tritici*)
[1] Loose Smut (*Ustilago tritici*)
[0] Flag Smut (*Urocystis agropyri*)
[2] Common Bunt (*Tilletia tritici* or T. *laevis*)
[0] Dwarf Bunt (*Tilletia controversa*)
[0] Karnal Bunt (*Tilletia indica*)
[0] Powdery Mildew (*Erysiphe graminis* f. sp. *tritici*)
[0] "Snow Molds"
[0] Common Root Rot (*Fusarium*, *Cochliobolus* and *Bipolaris* spp.)
[0] Rhizoctonia Root Rot (*Rhizoctonia solani*)
[0] Black Chaff (*Xanthomonas campestris* pv. *translucens*).
[0] Bacterial Leaf Blight (*Pseudomonas syringae* pv. *syringae*)
[0] Other (Specify) _____
[0] Other (Specify) _____
[0] Other (Specify) _____
[0] Other (Specify) _____

15. INSECT: (0 = Not Tested 1 = Susceptible 2 = Resistant 3 = Intermediate 4 = Tolerant)

PLEASE SPECIFY BIOTYPE (where needed)

[0] Hessian Fly (*Mayetiola destructor*)
[4] Stem Sawfly (*Cephus* spp.)
[0] Cereal Leaf Beetle (*Oulema melanopa*)
[0] Other (Specify) _____
[0] Other (Specify) _____
[0] Other (Specify) _____

Strongfield

15. **INSECT:** (continued) (0 = Not Tested 1 = Susceptible 2 = Resistant 3 = Intermediate 4 = Tolerant)

PLEASE SPECIFY BIOTYPE (Where Needed)

#200800079

[0]	Russian Aphid (*Diuraphis noxia*)	[0]	Other (Specify) _____
[0]	Greenbug (*Schizaphis graminum*)	[0]	Other (Specify) _____
[0]	Aphids	[0]	Other (Specify) _____

16. **ADDITIONAL INFORMATION ON ANY ITEM ABOVE, OR GENERAL COMMENTS:**

REPRODUCE LOCALLY. Include form number and edition date on all reproductions. FORM APPROVED - OMB No. 0581-0055

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE

EXHIBIT E
STATEMENT OF THE BASIS OF OWNERSHIP

Application is required in order to determine if a plant variety protection certificate is to be issued *(7 U.S.C. 2421)*. The information is held confidential until the certificate is issued *(7 U.S.C. 2426)*.

1. NAME OF APPLICANT(S)	2. TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER	3. VARIETY NAME
Agriculture and Agri-Food Canada	DT712	Strongfield
4. ADDRESS *(Street and No., or R.F.D. No., City, State, and ZIP, and Country)*	**5. TELEPHONE** *(Include area code)*	**6. FAX** *(Include area code)*
6000 C & E Trail Lacombe, Alberta T4L 1W1 Canada	(403) 782-8126	(403) 782-6120
	7. PVPO NUMBER 200800079	

8. Does the applicant own all rights to the variety? Mark an "X" in the appropriate block. **If no, please explain.** ☑ **YES** ☐ **NO**

9. Is the applicant (individual or company) a U.S. national or a U.S. based company? **If no, give name of country.** ☐ **YES** ☑ **NO**

CANADA

10. Is the applicant the original owner? ☑ **YES** ☐ **NO** **If no, please answer <u>one</u> of the following:**

a. If the original rights to variety were owned by individual(s), is (are) the original owner(s) a U.S. National(s)?
☐ **YES** ☐ **NO** **If no, give name of country**
CANADA

b. If the original rights to variety were owned by a company(ies), is (are) the original owner(s) a U.S. based company?
☐ **YES** ☐ **NO** **If no, give name of country**
CANADA

11. Additional explanation on ownership *(Trace ownership from original breeder to current owner. Use the reverse for extra space if needed)*:

Dr. John Clarke, the breeder of this durum wheat, was in the employ of Agriculture and Agri-Food Canada when the cross was made in 1994 and has been employed by Agriculture and Agri-Food Canada continuously since then. As a Crown employee, as per Section 3 of the Public Servants Inventions Act P-32 of Canada all inventions made by public servants acting within the scope of their dutes belong to Her Majesty the Queen in Right of Canada. Management of inventions owned by the Crown is delegated to government departments and to individuals who have been authorized to sign on behalf of the Crown.

PLEASE NOTE:

Plant variety protection can only be afforded to the owners (not licensees) who meet the following criteria:

1. If the rights to the variety are owned by the original breeder, that person must be a U.S. national, national of a UPOV member country, or national of a country which affords similar protection to nationals of the U.S. for the same genus and species.
2. If the rights to the variety are owned by the company which employed the original breeder(s), the company must be U.S. based, owned by nationals of a UPOV member country, or owned by nationals of a country which affords similar protection to nationals of the U.S. for the same genus and species.
3. If the applicant is an owner who is not the original owner, both the original owner and the applicant must meet one of the above criteria.

The original breeder/owner may be the individual or company who directed the final breeding. See Section 41(a)(2) of the Plant Variety Protection Act for definitions.

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

20080079

Research Branch | Direction générale de la recherché

Office of Intellectual Property & Commercialization
Agriculture and Agri-Food Canada

Commercialization Officer: Brenda Eamer
Brandon Research Centre
18th Street & Grand Valley Road
Brandon MB R7A 5Y3
Tel: (204) 578-3554
Fax: (204) 578-3589

February 11, 2008

Lead Scientist: J. Clarke
STAT # 456466

Dale Clark
Director of Research
Westbred LLC
81 Timerbline Drive
Bozeman, Montana
Email: dclark@westbred.com

This letter serves as confirmation that Agriculture and Agri-Food Canada (AAFC) is the sole owner of the Durum Wheat variety named Strongfield and that SeCan Association has been granted the sole license rights to commercialize Strongfield within the license territory of Canada and the United States. Under the terms and conditions of the License signed December 18, 2003 between SeCan and AAFC, SeCan has sub-licensed its license territory of the United States to Westbred LLC.

As the owner of the variety (Strongfield), AAFC gives Westbred LLC permission to apply for Plant Variety Protection on Strongfield on behalf of AAFC however AAFC shall be named as the owner and shall sign Section 25 of the Application for Plant Variety Protection Certificate, a copy of which is attached.

John Culley, Director
Office of Intellectual Property and Commercialization

/bee

Canada

U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
SCIENCE AND TECHNOLOGY
PLANT VARIETY PROTECTION OFFICE
BELTSVILLE, MD 20705

EXHIBIT F
DECLARATION REGARDING DEPOSIT

NAME OF OWNER (S)	ADDRESS *(Street and No. or RD No., City, State, and Zip Code and Country)*	TEMPORARY OR EXPERIMENTAL DESIGNATION
Agriculture and Agri-Food Canada	6000 C & E Trail Lacombe, Alberta T4L 1W1 Canada	DT712
		VARIETY NAME Strongfield
NAME OF OWNER REPRESENTATIVE (S)	**ADDRESS** *(Street and No. or RD No., City, State, and Zip Code and Country)*	[redacted]
Ann De St. Remy	6000 C & E Trail Lacombe, Alberta T4L 1W1 Canada	**PVPO NUMBER** 200800079

I do hereby declare that during the life of the certificate a viable sample of propagating material of the subject variety will be deposited, and replenished as needed periodically, in a public repository in the United States in accordance with the regulations established by the Plant Variety Protection Office.

Breeder: Dr. John Clarke
Contact Info: Semiarid Prairie Agricultural Research Centre
Po Box 1030
1 Airport Road
Swift Current, Saskatchewan
S9H 3X2
Canada
Phone (306)778-7221
Fax (306)778-3188

Signature

Feb 14, 2008
Date